abcam

Product datasheet

Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] ab283574

KO 評価済 リコンビナント RabMAb

2 References 画像数 13

製品の概要

製品名	Anti-COX2 / Cyclooxygenase 2 antibody [RM1026]
製品の詳細	Rabbit recombinant multiclonal [RM1026] to COX2 / Cyclooxygenase 2
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), ICC/IF, IP, IHC-P, WB **適用なし:** IHC-Fr
種交差性	**交差種:** Mouse, Rat, Human
免疫原	**This product was produced with the following immunogens:** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: U-87 MG, RAW 264.7, RAW 264.7 (treated with LPS), C6, C6 (treated with LPS) cell lysates, PTGS2 (COX2 / Cyclooxygenase 2) KO A549 (Human lung carcinoma epithelial cell) cell lysate, Wild-type A549 cell lysate, U-87 MG (Human glioblastoma-astrocytoma epithelial cell) whole cell lysate, MCF7 whole cell lysate. IHC-P: Human colon, Human colon carcinoma, Human liver tissues. ICC: RAW 264.7, U-87 MG cells. FC(Intra): RAW 264.7, U-87 MG cells. IP: U-87 MG, RAW 264.7 (treated with LPS) cells.
特記事項	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
バッファー	pH: 7.2

	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
精製度	Protein A purified
ポリ/モノ	Recombinant Multiclonal
クローン名	RM1026
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab283574の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

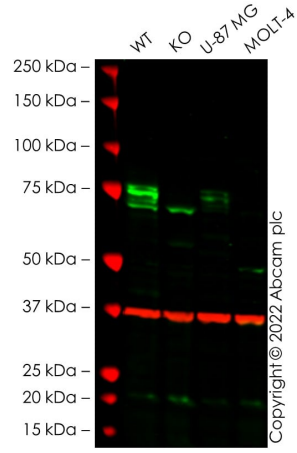
アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/50.
ICC/IF		1/500.
IP		1/30.
IHC-P		1/500.
WB		1/1000. Predicted molecular weight: 69 kDa.

追加情報 Is unsuitable for IHC-Fr.

ターゲット情報

機能	Mediates the formation of prostaglandins from arachidonate. May have a role as a major mediator of inflammation and/or a role for prostanoid signaling in activity-dependent plasticity.
パスウェイ	Lipid metabolism; prostaglandin biosynthesis.
配列類似性	Belongs to the prostaglandin G/H synthase family. Contains 1 EGF-like domain.
翻訳後修飾	S-nitrosylation by NOS2 (iNOS) activates enzme activity. S-nitrosylation may take place on different Cys residues in addition to Cys-561.
細胞内局在	Microsome membrane. Endoplasmic reticulum membrane.

画像



Western blot - Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] (ab283574)

All lanes : Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] (ab283574) at 1/1000 dilution

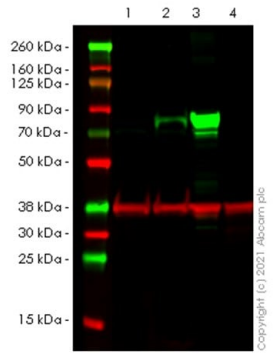
Lane 1 : Wild-type A549 cell lysate
Lane 2 : PTGS2 knockout A549 cell lysate
Lane 3 : U-87 MG cell lysate
Lane 4 : MOLT-4 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 69 kDa
Observed band size: 74-76 kDa

False colour image of Western blot: Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab283574 was shown to bind specifically to COX2 / Cyclooxygenase 2. A band was observed at 74-76 kDa in wild-type A549 cell lysates with no signal observed at this size in PTGS2 knockout cell line **ab280802** (knockout cell lysate **ab283825**). Band at 70 kDa in both wild-type and knockout samples is non-specific but exact protein is not determined. To generate this image, wild-type and PTGS2 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] (ab283574)

All lanes : Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] (ab283574) at 1/10000 dilution

Lane 1 : PTGS2 (COX2 / Cyclooxygenase 2) KO A549 (Human lung carcinoma epithelial cell) cell lysate with Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS
Lane 2 : Wild-type A549 cell lysate with Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS
Lane 3 : U-87 MG (Human glioblastoma-astrocytoma epithelial cell) whole cell lysate with Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS
Lane 4 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate with Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 69 kDa
Observed band size: 74 kDa

Negative control: MCF7 (PMID: 24325753, PMID: 16997132)

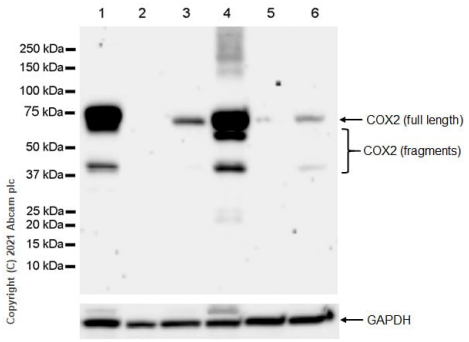
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

False colour image of Western blot: Anti- COX2 / Cyclooxygenase 2 antibody [RM1026] (ab283574) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red.

In Western blot, ab283574 was shown to bind specifically to COX2 / Cyclooxygenase 2. Target band was observed at 74 kDa in wild-type A549 cell lysates with no signal observed at this size in COX2 / Cyclooxygenase 2 knockout cell line **ab280802**. To generate this image, wild-type and COX2 / Cyclooxygenase 2 knockout A549 cell lysates were analyzed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes

were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] (ab283574)

All lanes : Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] (ab283574) at 1/1000 dilution

Lane 1 : U-87 MG (Human glioblastoma-astrocytoma epithelial cell) whole cell lysate
Lane 2 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate
Lane 3 : RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
Lane 4 : RAW 264.7 treated with 1 µg/ml lipopolysaccharide (LPS) for 6h whole cell lysate
Lane 5 : C6 (Rat glial tumor glial cell) whole cell lysate
Lane 6 : C6 treated with 100 ng/ml lipopolysaccharide (LPS) for 4h whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

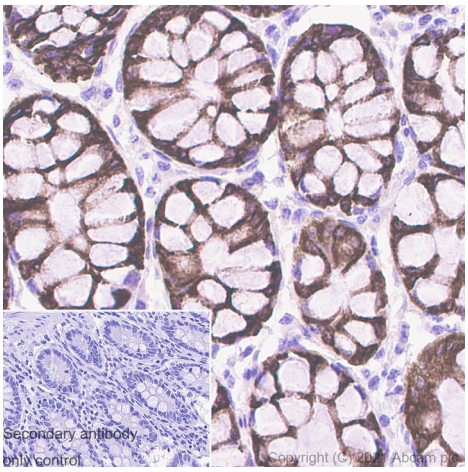
Predicted band size: 69 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Negative control: MCF7 (PMID: 24325753, PMID: 16997132)

Lower bands could be COX-2 fragments due to proteolysis. (PMID: 32366045)

Exposure time: Lane 1-4: 2 min Lane 5-6: 3 min

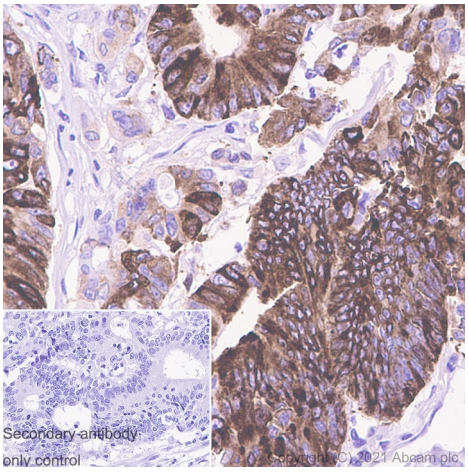


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] (ab283574)

Immunohistochemical analysis of paraffin-embedded human colon tissue labelling COX2 / Cyclooxygenase 2 with ab283574 at 1/500 dilution (1.058 µg/ml) followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer). Positive staining on human colon. The section was incubated with ab283574 overnight at 4°C. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer).

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0)

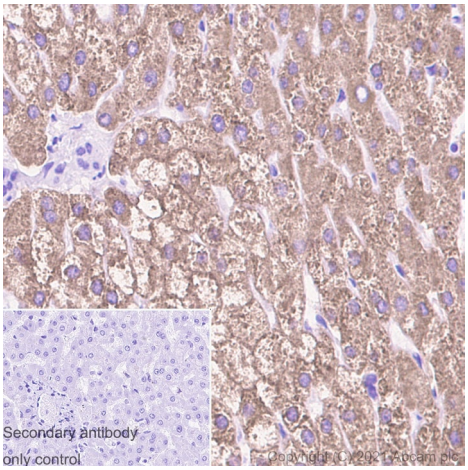


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] (ab283574)

Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue labelling COX2 / Cyclooxygenase 2 with ab283574 at 1/500 dilution (1.058 µg/ml) followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer). Positive staining on human colon carcinoma. The section was incubated with ab283574 overnight at 4°C. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer).

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0)

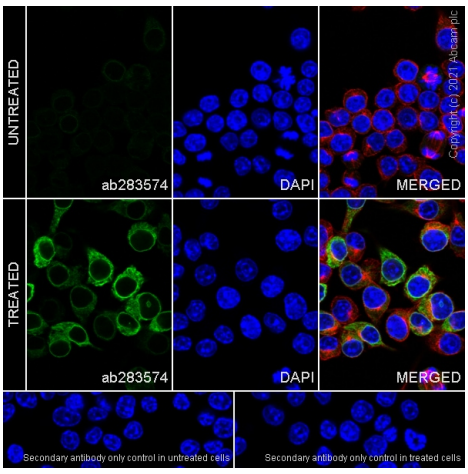


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] (ab283574)

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling COX2 / Cyclooxygenase 2 with ab283574 at 1/500 dilution (1.058 µg/ml) followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer). Positive staining on human colon liver. The section was incubated with ab283574 overnight at 4°C. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer).

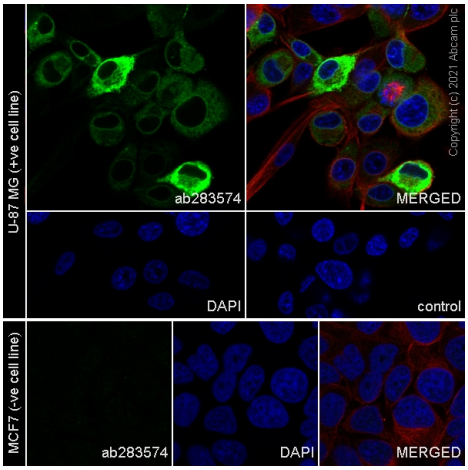
Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0)



Immunocytochemistry/ Immunofluorescence - Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] (ab283574)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) cells labelling COX2 / Cyclooxygenase 2 with ab283574 at 1/1000 dilution (0.529 µg/ml), followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (2 µg/ml)(Green). Confocal image showing cytoplasmic staining in RAW 264.7 cell line after treatment with lipopolysaccharide (1 µg/ml) for 6 hours is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (2.5 µg/ml)(Red). The Nuclear counterstain was DAPI (Blue).

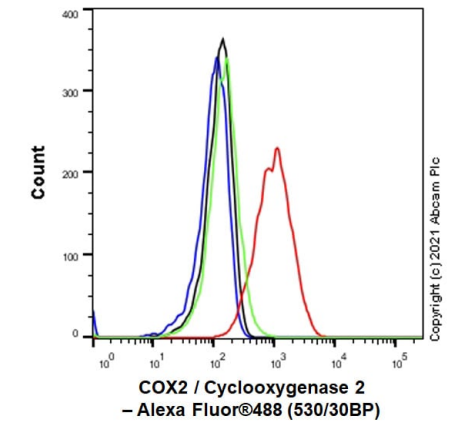
Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (2 µg/ml).



Immunocytochemistry/ Immunofluorescence - Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] (ab283574)

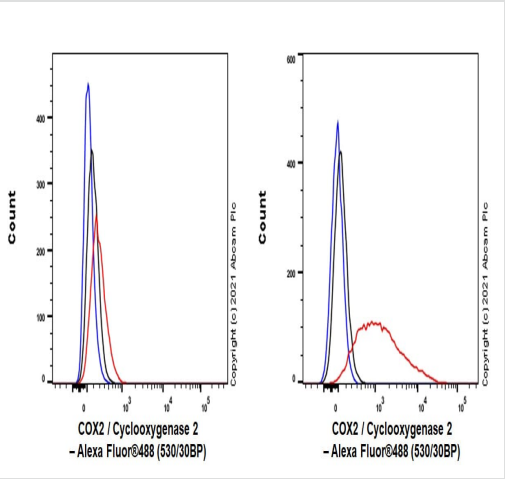
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized U-87 MG (human glioblastoma-astrocytoma epithelial cell) cells labelling COX2 / Cyclooxygenase 2 with ab283574 at 1/500 dilution (1.058 µg/ml), followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (2 µg/ml)(Green). Confocal image showing cytoplasmic staining in of U-87 MG cell line. **Negative control:** MCF7 (PMID:18199541) is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (2.5 µg/ml)(Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (2 µg/ml).



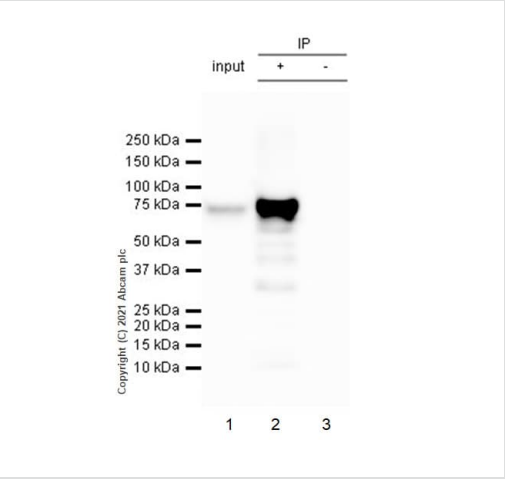
Flow Cytometry (Intracellular) - Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] (ab283574)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 1 µg/ml LPS for 6h (Red) / Untreated control (Green) cells labelling COX2 / Cyclooxygenase 2 with ab283574 at 1/500 dilution (0.1µg) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/500 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] (ab283574)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized MCF7 (human breast adenocarcinoma epithelial cell)(Left) / U-87 MG (human glioblastoma-astrocytoma epithelial cell)(Right) cells labelling COX2 / Cyclooxygenase 2 with ab283574 at 1/50 dilution (1µg) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/500 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] (ab283574)

COX2 / Cyclooxygenase 2 was immunoprecipitated from 0.35 mg U-87 MG (human glioblastoma-astrocytoma epithelial cell) whole cell lysate with ab283574 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab283574 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used at 1/5000 dilution.

Lane 1: U-87 MG (Human glioblastoma-astrocytoma epithelial cell) whole cell lysate 10µg

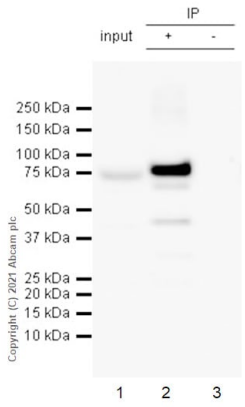
Lane 2: ab283574 IP in U-87 MG whole cell lysate

Lane 3:Rabbit monoclonal IgG (**ab172730**) instead of ab283574 in U-87 MG whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 6 seconds

Lower bands could be COX-2 fragments due to proteolysis. (PMID: 32366045)



Immunoprecipitation - Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] (ab283574)

COX2 / Cyclooxygenase 2 was immunoprecipitated from 0.35 mg RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 1 µg/ml LPS for 6h whole cell lysate with ab283574 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab283574 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (**ab131366**) was used at 1/5000 dilution.

Lane 1: RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 1ug/ml LPS for 6h whole cell lysate 10µg

Lane 2: ab283574 IP in RAW 264.7 treated with 1 µg/ml LPS for 6h whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab283574 in RAW 264.7 treated with 1 µg/ml LPS for 6h whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 6 seconds

Anti-COX2 / Cyclooxygenase 2 antibody [RM1026] (ab283574)

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